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A potassium transporter of the yeast *Schwanniomyces occidentalis* homologous to the Kup system of *Escherichia coli* has a high concentrative capacity

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The yeast *Schwanniomyces occidentalis* has a high-affinity K^+ uptake system with a high concentrative capacity, which is able to deplete the external K^+ to <0.03 μM. We have cloned the gene *HAK1* of *S.occidentalis* which complements defective K^+ uptake by *trk1* and *trk1 trk2* mutants of *Saccharomyces cerevisiae*. When HAK1 was expressed in a *trk1 trk2 S.cerevisiae* mutant, transport affinities for K^+ and other alkali cations resembled those of *S.occidentalis*. The predicted amino acid sequence of the HAK1 protein shows significant homology with the hydrophobic region of the Kup transporter of *Escherichia coli*. In *S.occidentalis* HAK1 expresses in K^+-limiting conditions. Our data indicate that in K^+-starved cells the system encoded by *HAK1* is the major K^+ transporter of *S.occidentalis*.

Key words: potassium transport/*Schwanniomyces occidentalis*/yeast

Introduction

In all living cells K^+ is the most abundant cation having concentrations ranging from 100 to 500 mM, except in some halophilic organisms, where its concentration can be much higher. Organisms living in environments diluted for K^+, such as soils and fresh water, must take up K^+ against very high transmembrane concentration gradients. In bacteria many transport systems for K^+ have been described (see Bakker, 1992), but in fungi and in the roots of plants, which probably use similar transport mechanisms, knowledge of the K^+ transport systems is limited. In fungi and plants a H^+-pumping ATPase is the primary source of energy for the transport of many nutrients (Serrano, 1989; Goffeau and Green, 1990). In response to the membrane potential created by the H^+ pump, K^+ uptake can be mediated by inward-rectifying K^+ channels (Schroeder *et al.* 1994). However, the concentrative capacity of a channel is not sufficient to explain K^+ uptake in media depleted of K^+ (Maathuis and Sanders, 1993). In these cases, highly concentrative K^+ uptake has been shown to occur by coupling the movements of K^+ with other cations, thus giving rise to a process that significantly increases the driving force of the membrane potential. In *Neurospora crassa* (Rodríguez-Navarro *et al.* 1986; Blatt *et al.* 1987) and *Arabidopsis thaliana* (Maathuis and Sanders, 1994) the coupled ion is H^+, and in *Chara australis* it is Na^+ (Smith and Walker, 1989; McCulloch *et al.*, 1990). The existence of a K^+-ATPase has been hypothesized in plants (see Kochian *et al.*, 1989), but has never been demonstrated.

Saccharomyces cerevisiae is the only fungus from which genes encoding K^+ transporters have been isolated. K^+-starved cells of this species can concentrate K^+ to ~10^5 times the level found in the external medium, exhibiting a K_m for K^+ influx of 10–20 μM (Rodríguez-Navarro and Ramos, 1984). The K^+ uptake system is probably composed of several different components (Ramos *et al.*, 1994). The genes of two of these components, *TRK1* (Gaber *et al.*, 1988) and *TRK2* (Ko and Gaber, 1991), have been cloned. TRK2 has a high degree of identity with TRK1 though missing 314 amino acids between the M3 and M4 transmembrane regions and may have evolved through gene duplication. The existence of TRK2 became apparent in K^+ uptake studies of a *trk1* mutant. *TRK2* encodes a system of moderate affinity for K^+ and Rb^+ (K_m of 0.3 mM Rb^+), whereas *trk1 trk2* null mutants are extremely deficient in K^+ uptake, exhibiting K_ms of 60 mM Rb^+ (Ramos *et al.*, 1994). In plants, three K^+ transport genes have been studied: *KAT1* (Anderson *et al.*, 1992; Schachtman *et al.*, 1992), *AKT1* (Sentenac *et al.*, 1992), and *HKT1* (Schachtman and Schroeder, 1994). *KAT1* and *AKT1* encode K^+ channels, which cannot account for a highly concentrative process (Maathuis and Sanders, 1993), and *HKT1* encodes a transporter with high affinity for K^+, but the capacity of this transporter to accumulate K^+ has not been reported.

Schwanniomyces occidentalis is an ascomycete yeast isolated from soils (Phaff, 1970) with an ability to grow in nutritionally limiting conditions. Several genes from this organism have been expressed in *S.cerevisiae* (Klein and Favreau, 1988; Klein and Roof, 1988; Claros *et al.*, 1993). We have isolated a gene (*HAK1*) from *S.occidentalis* which complements *trk1*Δ and *trk1*Δ *trk2*Δ mutant strains of *S.cerevisiae*. *S.cerevisiae* expressing HAK1 can accumulate K^+ to transmembrane ratios higher than 10^6.

Results

K^+ uptake in *S.occidentalis*

In AP medium with arginine as the sole nitrogen source and K^+ as the limiting nutrient, *S.occidentalis* depleted K^+ in the external medium to <0.03 μM K^+. The cells contained 1.5 μl of H_2O/mg (dry weight) and 300 nmol K^+/mg, thus giving an extremely high internal/external transmembrane gradient of K^+. In general, the kinetic characteristics of the K^+ uptake system of *S.occidentalis* were very similar to those found in *S.cerevisiae*, in which

Table I. Apparent affinities of alkali cations for the transport system of *S.occidentalis*

Type of cells	K_m (mM)		
	K^+	Rb^+	Li^+
Normal K^+ cells[a]	4[b]	4[b]	20[e]
K^+-starved cells[c]	0.001[d]	0.001[d]	12[e]

[a]Cells grown in AP medium with 3 mM K^+, containing 850 nmol K^+/mg, centrifuged and suspended in testing buffer.
[b]Rb^+ influx cannot be tested in the complete absence of K^+ because, in this case, the time-course of Rb^+ uptake is not linear. Values were obtained by testing Rb^+ influxes in different concentrations of K^+, and calculating the K_m and K_i for both cations from the competitive inhibition equation by regression analysis.
[c]Cells grown in AP medium with 3 mM K^+, incubated for 4 h in K^+-free AP medium, transferred to testing buffer, and incubated for 10 min before starting the experiment to deplete the contaminating K^+. Cells containing 280 nmol K^+/mg (see Figure 3).
[d]Tests of Rb^+ influxes in the absence of K^+, and in the presence of different concentrations of K^+ to calculate the K_i for K^+. Technical difficulties made it impossible to test the uptake in <10 μM Rb^+, the K_m values are approximate (between 0.0005 and 0.002 mM).
[e]Calculated from Li^+ influxes in the absence of K^+, in cells prepared as described for Rb^+ uptake experiments.

the K_ms for the alkali cations vary with the K^+ content of the cells (Ramos and Rodríguez-Navarro, 1986). Normal-K^+ cells showed high K_ms for K^+ and Rb^+ and low discrimination against Li^+, whereas K^+-starved cells showed low K_ms for K^+ and Rb^+ with higher discrimination against Li^+. The most striking differences with *S.cerevisiae* were the extremely low K_m for K^+ (1 μM, approximately) and the lack of discrimination between K^+ and Rb^+ (Table I). Southern blot analysis of *S.occidentalis* genomic DNA probed with the 2.3 kb *Xba*I fragment of the *TRK1* gene of *S.cerevisiae* did not reveal the presence of any gene homologous to *TRK1*.

Cloning and sequence analysis of HAK1

A *S.cerevisiae* strain carrying a null mutation in the *TRK1* gene (strain TE12) was transformed with a genomic library of *S.occidentalis* inserted in the episomal plasmid pYcDE8 (Klein and Favreau, 1988), and transformants plated on KNa medium containing 0.3 mM K^+. Several transformants isolated on this medium had the same plasmid, pAG22, which contained a 9 kb insert. The plasmid pAG22, when introduced into *trk1*Δ mutants conferred the ability to grow on KNa medium with 0.3 mM K^+. A plasmid, pAG5 containing a 5.0 kb *Bam*HI fragment isolated from pAG22 was sufficient to complement the *trk1* mutation with the same efficiency as pAG22. The putative gene contained within the 5.0 kb *Bam*HI fragment, responsible for complementing the *trk1* mutation was called *HAK1* (High Affinity K^+ transporter). Southern blot analysis of *S.occidentalis* genomic DNA probed with a 2.1 kb *Eco*RI fragment isolated from pAG5 (Figure 1) indicated that the *HAK1* gene was present probably in only one copy in the genome of *S.occidentalis*. Northern blot analysis (Figure 2) indicated that the *HAK1* gene is expressed as a transcript of ~2600 nucleotides. The HAK1 mRNA was detected only in K^+-starved cells, suggesting that the expression of the gene is probably very low when the cells do not require a highly active K^+ transporter. The transcript was also detected in strain 9.3 of *S.cerevisiae*

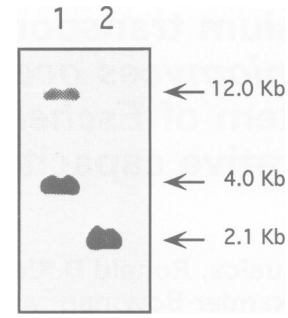

Fig. 1. Probing of total DNA from *S.occidentalis* with a digoxigenin-labelled 2.1 kb *Eco*RI fragment isolated from pAG5. *Xba*I (lane 1) and *Eco*RI (lane 2) digested DNA (3 μg) fractionated through a 0.5% agarose gel, transferred to a nylon membrane (Hybond-N, Amersham) and probed with the 2.1 kb *Eco*RI fragment. In the restriction map of pAG5 *Xba*I splits the *Eco*RI fragment in two fragments of 1.7 and 0.4 kb.

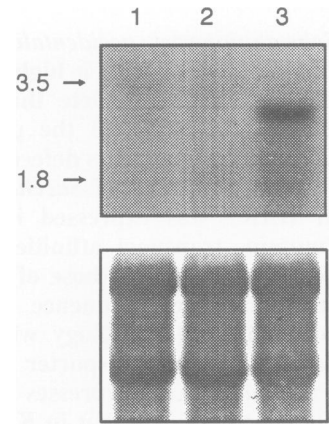

Fig. 2. Northern analysis of the *HAK1* transcripts in *S.occidentalis*. Total RNA extracted from cells grown on YPD medium (lane 1), AP medium with 3 mM K^+ (lane 2), and cells grown in AP medium with 3 mM K^+ and then K^+-starved for 3 h (lane 3). Top panel: 20 μg of RNA were fractionated through formaldehyde agarose gels, transferred to a nylon membrane (Hybond-N, Amersham) and probed with the 2.1 kb *Eco*RI fragment isolated from pAG5; the position of the ribosomal RNAs of 3.5 and 1.8 kb is indicated at the left. Bottom panel: the filter was stripped and stained with 0.04% methylene blue in 0.5 M sodium acetate (pH 5.2) as a control.

transformed with plasmid pAG5, both in K^+-starved cells and in cells growing at 50 mM K^+. The level of expression detected in the former was lower than that found in K^+-starved cells of *S.occidentalis*, and in the latter the HAK1-specific transcript was at the limit of detection.

DNA sequence analysis of the entire 5.0 kb *Bam*HI fragment of pAG5 revealed an open reading frame (ORF) of 2286 nucleotides, encoding a polypeptide of 762 amino acids. A hydrophilicity profile of the deduced polypeptide (Kyte and Doolittle, 1982) predicted 12 membrane-spanning regions in the first 470 amino acids. Overall, the HAK1 protein has 60% similarity and 32% identity to the *kup* gene product (Schleyer and Bakker, 1993) from *E.coli*. The most remarkable homology between the two proteins is in the putative membrane-spanning regions (479 amino acids of HAK1 and 440 of Kup), which show a 64% similarity and 35% identity. The most favourable alignment of these regions between the two proteins (Figure 3) required the introduction of two gaps of 28 and 21 amino acids in the Kup sequence between transmembrane regions

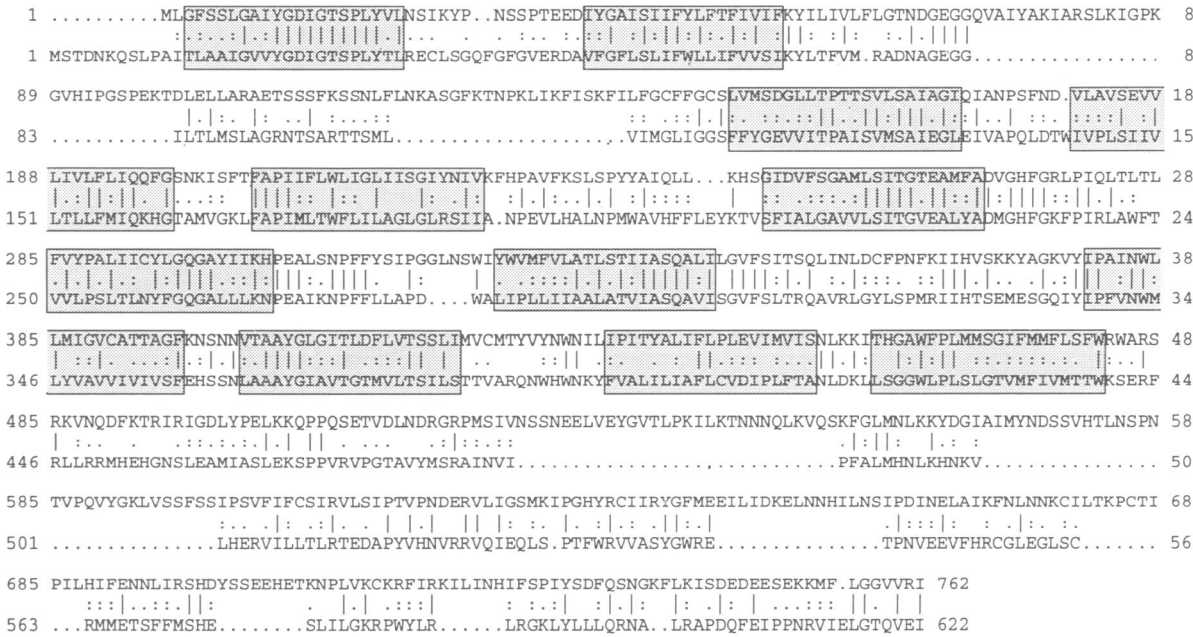

Fig. 3. Alignment of the deduced amino acid sequence of the translated ORF of *HAK1* (top lines in the alignment) with the Kup K^+ transporter of *E.coli* (Schleyer and Bakker, 1993). The putative transmembrane regions are shown in grey boxes.

M_2 and M_3, and three gaps of minimal significance (two in HAK1, and one in Kup). The minimal consensus sequence found in ABC transporters, GKT(S) (Higgins *et al.*, 1986), was not present in the translated ORF of *HAK1*.

HAK1 mediates 'active' K^+ uptake in *S.cerevisiae*

HAK1 complemented *trk1* or *trk1 trk2* mutant strains of *S.cerevisiae* when expressed on multicopy, episomic plasmids (pAG22 and pAG5), conferring to these strains the ability to take up K^+ at levels below 2 μM, which is the limit for wild-type strains of *S.cerevisiae*. Similar constructs using low copy, centromeric plasmids (*HAK1* inserts from pAG22 and pAG5 cloned in plasmids pRS414 and YCp50, respectively) resulted in less efficient complementation, for example, *HAK1* transformants of the *trk1* strain required 1 mM K^+ in KNa medium.

Figure 4 shows the comparison of K^+ uptake in a *trk1 trk2* double mutant (strain 9.3) containing pRH22 (*TRK1*) (this transformant is indistinguishable from wild-type *S.cerevisiae*), or pAG22 (*HAK1*), and a wild-type strain of *S.occidentalis*. The mutant transformed with HAK1 (Figure 4A) depleted K^+ from the external medium to almost the same extent as seen with the wild-type strain of *S.occidentalis* (Figure 4C), though the activation of HAK1 was delayed for ~2 h. After activation the depletion of K^+ was more rapid and reached a lower level in this transformant than in the transformant with the *TRK1* gene (Figure 4B). Within 6 h the *HAK1* transformant reached 0.1 μM K^+ and the *TRK1* transformant 3 μM K^+; the internal K^+ content of the cells was similar in both strains (300 nmol K^+/mg). The wild-type strain of *S.occidentalis* showed a more rapid decrease of the external K^+, but also had the greatest change in the internal K^+.

Kinetics of Rb^+ influx

In the *trk1 trk2* strain containing pAG22 expressing HAK1, grown in 50 mM K^+, a concentration that allows rapid growth of the mutant, the kinetics of Rb^+ influx was distinctly biphasic (Figure 5A). This suggested the existence of two systems: a low-affinity system, exhibiting the 60 mM Rb^+ K_m, characteristic of the host *trk1 trk2* strain (Ramos *et al.*, 1994, see also Figure 5A), and a system with a K_m of 2–5 mM Rb^+. This latter system is not found in the host strain, and must be the result of the presence of *HAK1*. K^+ inhibited Rb^+ uptake competitively. However, because Rb^+ uptake could not be tested in the complete absence of K^+, as described previously (Rodríguez-Navarro and Ramos, 1984), and the V_{max} of Rb^+ influx was low (1.5 nmol Rb^+/mg/min), it was not possible to calculate the exact K_i for K^+ influx, and the K_m for Rb^+. Li^+ and Cs^+ inhibited competitively Rb^+ uptake mediated by HAK1 with K_is of 20–30 mM Li^+ and 5–10 mM Cs^+.

A high-affinity uptake was seen in Rb^+ influx studies (Figure 5B) in cells starved for 6 h in low K^+ medium. This high-affinity uptake is not present in the *S.cerevisiae trk1 trk2* mutant. Competition experiments of Rb^+ influx with K^+ showed that the K_i for K^+ and the K_m for Rb^+ were both 5 μM. The Li^+ K_i on Rb^+ influx (14 mM Li^+) showed no significant change after starving the cells for 6 h in low K^+. On the contrary, this starvation significantly decreased the K_i for Cs^+ (30–60 μM). Starving the cells of K^+ for increasing periods of time showed that the K_m of Rb^+ influx through the HAK1 system decreased continuously from the level in cells with a normal K^+ content (2–5 mM Rb^+) up to the value found in K^+-starved cells (5 μM Rb^+).

Arrhenius plots of Rb^+ influxes

The Arrhenius plots of Rb+ influx in *S.cerevisiae* (Rodríguez-Navarro and Ramos, 1984), *N.crassa* (Rodríguez-Navarro and Ramos, 1986), and *Helianthus annus* (sunflower plant) (Benlloch *et al.* 1989) are very similar. In the three species, K^+ starvation changes the plots observed in cells or plants with a normal K^+ content.

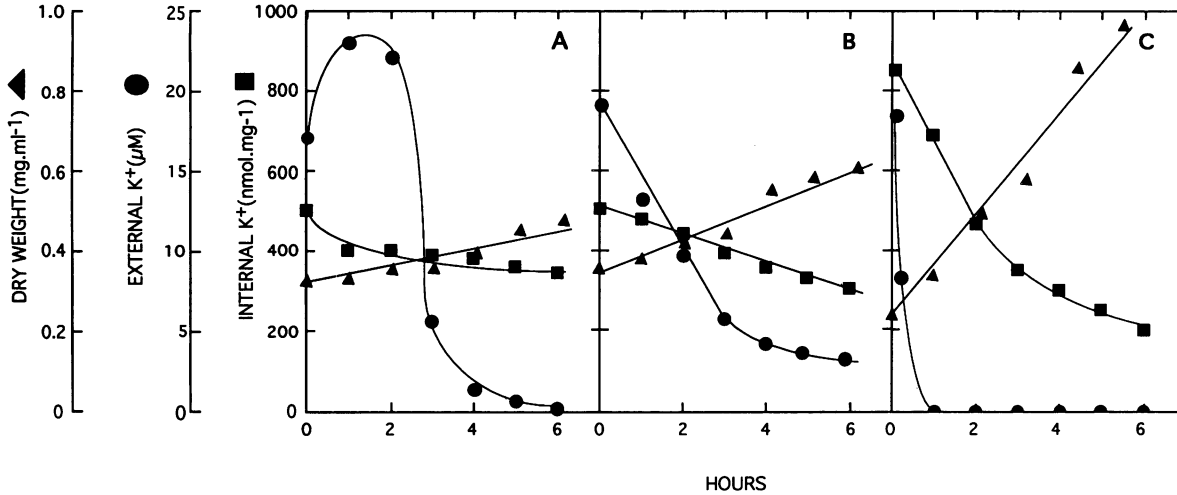

Fig. 4. Growth response, and changes in internal and external K^+ in *S.cerevisiae* strain 9.3 (*trk1*Δ *trk2*Δ mutant) expressing either HAK1 (**A**) or TRK1 (**B**), and in *S.occidentalis* (**C**). Cells were grown in AP medium supplemented with 50 mM KCl and transferred to fresh medium containing 15 μM K^+. At the times indicated, samples were removed and internal and external K^+ concentrations and cell densities determined.

These characteristics have been taken as evidence of homologous systems for K^+ uptake. Because HAK1 is significantly different from TRK1, we tested the effect of temperature on Rb^+ influx mediated by HAK1, in cells with a normal K^+ content, and in K^+-starved cells. To reduce as much as possible the effect of the endogenous system in the *trk1 trk2* mutant, normal-K^+ cells were grown in 3 mM K^+ in PA medium, and Rb^+ influx was tested at 5 mM Rb^+. The Arrhenius plots for these studies were essentially as previously described for *S.cerevisiae*, a straight line for cells with a normal-K^+ content, and a plot with a break point at 30°C exhibiting decreasing rates with increasing temperatures above 30°C.

HAK1 is activated by glucose and inhibited by neomycin

The activation and inactivation of TRK1 by various conditions and compounds has been reported (Alijo and Ramos, 1993). We have studied the effect of glucose derepression and neomycin in the *trk1 trk2 S.cerevisiae* strain 9.3 containing pAG22 expressing HAK1. Glucose activated Rb^+ influx mediated by HAK1 as it does in the case of TRK1 (Ramos *et al.*, 1992) by increasing the V_{max}. Neomycin (2 mM), however, showed a differential effect in both systems, it inhibited HAK1-mediated influx by 75%, but did not have any effect on strain 9.3 containing pRH22 expressing TRK1.

Discussion

We have cloned a gene from *S.occidentalis* (*HAK1*) which encodes a K^+ transporter, that has significant amino acid homology with the *kup* gene product of *E.coli* (Schleyer and Bakker, 1993). The expression of HAK1 is probably regulated by the level of K^+ and in K^+-starved cells may constitute the major K^+ transport system in *S.occidentalis*.

Expression of HAK1 in a *trk1 trk2* mutant of *S.cerevisiae* allowed the functional characterization of the gene. This mutant has an endogenous low-affinity K^+ uptake system (K_m for Rb^+ of 60 mM) that could be distinguished from K^+ uptake mediated by HAK1 (K_m for Rb^+ of 2–5 mM in cells grown at 50 mM K^+ and 5 μM in K^+-starved cells) (Figure 5). HAK1 expressed in the *trk1 trk2* mutant of *S.cerevisiae* showed a remarkable feature characteristic of K^+ uptake in *S.occidentalis*, namely the capacity to concentrate K^+ at well over 10^6, which is unusual for a non-ATPase K^+-uptake system. The most significant difference between HAK1 expressed in *S.cerevisiae* and K^+ uptake in *S.occidentalis* is the higher K_m for Rb^+ and K^+ in *S.cerevisiae*, 5 μM versus 1 μM. Although the existence of another uptake system in K^+-starved cells of *S.occidentalis* cannot be ruled out, it is more likely that HAK1 does not reach its lowest K_m in *S.cerevisiae* because of defective regulation. Defective regulation may also explain the delayed activation of uptake shown in Figure 3. Conclusions regarding the comparison of Kup and HAK1 are difficult to assess. For example, the Kup system has a higher K_m for K^+ (0.37 mM) (Bossemeyer *et al.*, 1989; Dosch *et al.*, 1991). However, this K_m was from

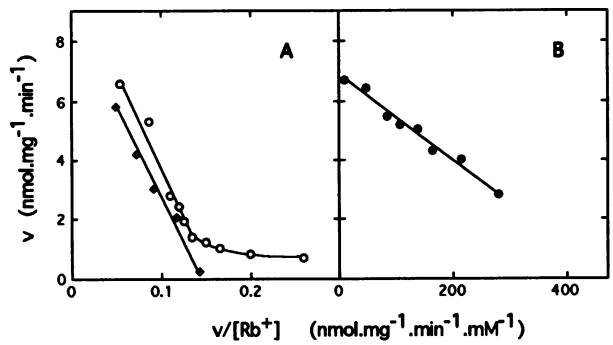

Fig. 5. Eadie–Hofstee plots of Rb^+ influx in cells of *S.cerevisiae* strain 9.3 (*trk1*Δ *trk2*Δ mutant) expressing HAK1. (**A**) Rb^+ influx in cells with a normal K^+ content (○); the panel also shows the plot of Rb^+ influx in a non-transformed 9.3 strain (◆). (**B**) Rb^+ influx in K^+-starved cells (●). Normal-K^+ cells were grown in AP medium supplemented with 50 mM KCl to a cell density of 0.2–0.3 mg/ml, harvested, and resuspended in testing buffer containing 0.5 mM K^+, because, in these cells, the time-course of Rb^+ uptake is not linear in the complete absence of K^+. To prepare K^+-starved cells, normal-K^+ cells were harvested, washed and transferred to K^+-free AP medium. After 6 h the cells were centrifuged and resuspended in testing buffer. Because the suspensions of cells contained K^+, the K^+ content was standardized to 8 μM. For technical reasons the lowest Rb^+ concentration tested was 10 μM.

cells depleted of K^+ by chemical treatment, and K^+ starvation during growth, as used in this report, may decrease the K_m with reference to chemical depletion, as in the case of the TRK1 system of *S.cerevisiae* (Ramos and Rodríguez-Navarro, 1986).

The mechanism that allows a transport system to vary the K_m 1000-fold for K^+ and Rb^+ with minimal changes in the K_ms of the smaller ion Li^+ is remarkable. The physiological importance of this response, regarding Na^+ and Li^+ tolerances, has been already shown (Haro *et al.*, 1993), but the mechanism that mediates this change is unknown. HAK1 and TRK1 respond in the same manner, but they present a substantial difference. In TRK1 the discrimination between K^+ and Rb^+ increases 10 times in the process of acquiring the high-affinity state (Ramos *et al.*, 1985), and in HAK1 that discrimination does not exist in any state. It is interesting that, in *S.cerevisiae*, the regulatory pathway that mediates these changes in TRK1 also acts on HAK1 with minimal defects. Furthermore, in *S.cerevisiae* HAK1 is activated by glucose, and it may be assumed that this occurs by the same mechanism activating the K^+ uptake system of *S.cerevisiae* (Alijo and Ramos, 1993). In contrast, neomycin has opposite effects on TRK1 and HAK1, indicating differences in the regulation of these systems by phosphatidylinositol signalling pathways.

Regarding the concentrative capacity of HAK1, there is no doubt that it is greater than expected if K^+ moves through a uniporter or channel. Considering water content, large size vacuoles and K^+ content in both vacuoles and cytoplasm of K^+-starved cells of *S.cerevisiae* (Ramos *et al.*, 1990) it is clear that K^+ is not <190 mM in the cytoplasm versus 0.1 μM K^+ in the external medium (Figure 3). This gives a K^+ diffusion potential of not less than –370 mV, which is very likely negative to the actual membrane potential of *S.cerevisiae*. In *S.occidentalis* the concentrative capacity is even greater, but the existence of an additional K^+ uptake system, although unlikely, cannot be ruled out. The mechanism involved in this highly concentrative process cannot be deduced from the putative peptide sequence of HAK1, which only shows that HAK1 is not a P-type ATPase nor an ABC transporter. The significant homology of the deduced HAK1 polypeptide with the Kup system of *E.coli* or the similarities with the kinetics of TRK1 K^+ uptake are not informative since the mechanisms of these two systems are unknown. TRK1 has been suggested to be an ATPase (Kochian *et al.*, 1989; Fernando *et al.*, 1992). However, TRK1 does not present homology with P-type ATPases, and the nucleotide-binding domain of TRK1 (Gaber *et al.*, 1988) only may suggest that TRK1 is a component of an ABC transporter (Hyde *et al.*, 1990).

It has been hypothesized that two independent systems for K^+ uptake exist in plants (see for example, Fernando *et al.*, 1992). According to this hypothesis, high-affinity and low-affinity transporters account for uptake in plants with low and high K^+ status, respectively (the low-affinity transporter in plants with a high K^+ content should not be confused with the low-affinity component exhibited in the kinetics of K^+ influx in low-K^+ plants, see Benlloch *et al.*, 1989). Present results indicate that the number of systems cannot be established only on the basis of kinetic differences. In a *trk1 trk2* mutant HAK1 mediates growth in millimolar concentrations of K^+, with influxes exhibiting low-affinity K_ms (growth in 1–5 mM K^+; K_m for K^+, 1–10 mM), and also growth in micromolar concentrations of K^+, with influxes exhibiting high-affinity K_ms (growth in 10–100 μM K^+; K_m for K^+, 10–100 μM). Therefore, low-affinity and high-affinity uptake is mediated only by HAK1.

The similarities in the Arrhenius plots of the Rb^+ influxes in *S.cerevisiae*, *N.crassa*, and *H.annus* led to the suggestion that the same system may operate in these species (Benlloch *et al.*, 1989). The same type of plots, typical after K^+ starvation, have now been found when Rb^+ influx is mediated by HAK1. The absence of peptide sequence identity and similarity between HAK1 and TRK1 discounts potential structural homology as an explanation for the similarities of these plots. Because the Arrhenius plots of H^+ pumping and maltose–H^+ symport do not show the characteristics of the plots of Rb^+ uptake (J.Ramos and A.Rodríguez-Navarro, unpublished results), it seems that K^+ uptake through different systems has common features. These systems may operate in two different modes, depending on whether the K^+ content is normal or low. The break point with decreasing rates above the cardinal temperature when the K^+ content of the cells is low may occur if K^+ uptake shows voltage sensitivity in the physiological range of voltage, as is the case with *N.crassa* (Blatt *et al.*, 1987). Then the decreasing rates with the increase in temperature, above a certain temperature, would reflect depolarization induced by an increase in permeability.

The functional expression of HAK1 in a *trk1 trk2* mutant of *S.cerevisiae* strongly suggests that HAK1 is a K^+ transporter or a component of it. The possibility that HAK1 is a regulator not involved in K^+ uptake but functionally activates a transporter of higher affinity and higher concentrative capacity than TRK1 is unlikely. TRK1 may be a component of a more complex system (Ramos *et al.*, 1994); however, the absence of sequence homology between TRK1 and HAK1 makes it unlikely that HAK1 substitutes for TRK1 in such a complex. In *E.coli* (Dosch *et al.*, 1991) Kup functions as a K^+ transporter and is not a component of a more complex system. HAK1 has significant homology with Kup. Taken together, these observations suggest that HAK1 is a K^+ transporter.

Materials and methods

Strains, methods and media

The *S.cerevisiae* strain TE12 (*Matα trk1 ura3 his3 trp1 ena1::LEU2::ena4*) has been described previously (Haro *et al.*, 1993); strain 9.3 (*Mata trk1 trk2 ura3 leu2 trp1 ade2 ena1::HIS3::ena4*) was constructed by crossing strains W59, G19 and M469. W59 (*Mata ura3 his3 trp1 ade2 trk1::LEU2*) and G19 (*Matα ura3 his3 trp1 ade2 ena1Δ::HIS3::ena4Δ*) were obtained by gene disruption from the laboratory strains W303.1A and W303.1B, respectively, and strain M469 was a *trk2::HIS3* mutant obtained from Richard Gaber. The *S.occidentalis* strain used in this study was ATCC 26076 and has been described previously (Deibel *et al.*, 1988; Klein and Roof, 1988). The strains were routinely grown in YPD medium (1% yeast extract, 2% peptone, 2% glucose). The K^+- and Na^+-free minimal media used in this study were modifications of the synthetic minimal (SD) medium described by Sherman (1991) with either ammonium–phosphate (KNa medium) or arginine neutralized with phosphoric acid (AP medium) as nitrogen sources as reported before (Rodríguez-Navarro and Ramos, 1984). Media supplemented with KCl are indicated in the text. Growth temperature was 28°C.

DNA manipulation and sequence analysis

Manipulation of nucleic acids was by standard protocols (Sambrook *et al.*, 1989) or, where appropriate, following the manufacturer's instructions. Southern hybridization analysis was carried out using digoxigenin-labelled probes as per the manufacturer's instructions (Boehringer Mannheim, Bad Widbad, Germany). For Northern blots of *HAK1* total RNA was extracted (Carlson and Botstein, 1982), fractionated through formaldehyde gels, and transferred to membranes. DNA fragments used for probes were labelled by the random priming method (Feinberg and Vogelstein, 1983). The *S.occidentalis* genomic library used in this study has been described previously (Klein and Favreau, 1988). DNA sequence analysis and data processing were as described (Klein *et al.*, 1989). DNA sequence data for comparative analysis were obtained from GenBank (release 83 [6/94]). Peptide sequences were obtained from GenBank or SwissProt (release 83 [3/94]). Protein comparisons were performed using the BESTFIT, GAP, and FastA (updated FastP) algorithms from the University of Wisconsin Computer Group (Devereux *et al.*, 1984 and updates). The GenBank accession number for *HAK1* sequence is U22945.

Cation contents and fluxes

Cells were collected on Millipore membrane filters, rapidly washed with 20 mM $MgCl_2$ solution, acid extracted and analysed by atomic emission spectrophotometry. Uptake experiments were carried out in 10 mM morpholinoethanesulphonic acid brought to pH 6.0 with $Ca(OH)_2$, containing 0.1 mM $MgCl_2$ and 2% glucose (testing buffer). Cells prepared as described in each case were suspended in testing buffer pre-equilibrated at the required temperature. The initial rates of Rb^+ uptake (Rb^+ influx) were determined from the time courses of the cellular Rb^+ content, and reported as the means from at least four independent experiments. Except in K^+-starved cells of *S.occidentalis* Rb^+ uptake has to be tested in the presence of K^+ concentrations that produce significant inhibitions. Therefore, the apparent K_ms for Rb^+ at several concentrations of K^+ were used to calculate the K_m for Rb^+ and the K_i for K^+ from the competitive inhibition equation by regression analysis. Cellular water content was determined as previously described (Ramos *et al.*, 1990), by labelling the total water with ^3H_2O and the extracellular water with D-[U-^{14}C]sorbitol. Results are reported as the means of at least four independent experiments. The standard errors of the means were lower than 20% of the corresponding mean, except when minimal and maximal values are presented.

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